REMARKS

Claims 1-45 and 49-50 are the elected Group I claims and claims 46-48 are the non-elected Group II claims. For ease of examination, the Examiner has requested that individual species in Group I be elected. However, in the event that a generic claim is allowable, the non-elected species will be rejoined. The non-elected species in Group I are claims 14-28 and 34-39. Claims 1-13, 29-33, 40-45, 49 and 50 are pending. The Examiner has objected to claims 3-5, 31-32 and 42. Claims 1, 3-5, 31-32 and 42 have been amended.

Claims 3 to 5 further limit the subject matter of claim 1 and are therefore properly dependent on claim 1. Claims 3 to 5 further define the target nucleic acid in claim 1 element (a) and (e) and are amended accordingly.

Rejection under 35 U.S.C. §112

Claims 31 and 32 have been amended so as to correct the insufficiency of antecedent basis. The Examiner is requested to reverse the rejection. Claim 1 has been amended. Support for the amendment can be found in Figure 2 and Figure 3.

Rejection under 35 U.S.C. §§102(b) and (e)

Claims 1-13, 29-33 and 40-45 require that the duplex nucleic acid be unwound by contacting the duplex with a helicase preparation.

Applicants have amended the claims to specify in claim 1, the particular embodiments provided on page 22 of the application.

The Examiner has rejected the claims as anticipated or obvious in view of Sninsky et al. (U.S. Patent No. 5,176,995) or Dean et al. U.S. Patent No. 6,977,148). Neither of these references suggest or teach the use of a helicase preparation that comprises a helicase and single strand binding protein unless the helicase is thermostable in which case, the single strand binding protein is optional. Neither of the references are enabled for helicase-dependent amplification.

Sninsky reference

The Examiner has pointed out numerous cites to the text of the references in support of the rejection. For Sninsky et al., the Examiner has cited the following: col. 4, lines 34-40, col. 5, lines 61-68, col. 6 lines 61-68, cols. 7 and 8, col. 9, lines 1-64, col. 11, lines 13-20, lines 20-22, col. 12, lines 24-31, 50-64, col. 13, lines 46-53, col. 14, lines 18-23, 35-54, col. 15,

lines 10-18, col. 17, lines 45-50, col. 18, lines 40-49, and col. 20, lines 51-62.

Most of these cites are background descriptions covering prior art aspects of PCR amplification with particular reference to viruses. Within the general description of PCR amplification, Sninsky et al. describes how denaturing DNA prior to amplification can be achieved not only by a change in temperature (physical means) but also by chemical and enzymatic means. The Sninsky reference then recites that strand separation can be accomplished by using a helicase alone or by a recA (which is not a helicase) according to the methods of Kuhn Hoffman-Berling or Radding. (Col. 12, lines 40-50). However, neither of these references enable the present claimed methods. The Sninsky reference further mentions helicase in column 14, lines 46-50. However, this cite again does not describe the required combined use of a single strand binding protein and a helicase. Thermostable helicases were not known at the time the application was filed so this cite must be interpreted as referring to mesophilic helicases only. Mesophilic helicases require single strand binding proteins to enable amplification.

<u>The Sninsky reference is not enabled for the use of helicases in amplification</u>

The reference teaches only that:

Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or the

> enzyme recA which has helicase activity and in the presence of riboATP is known to denature DNA. (col. 12, lines 40- 44)

The reference incorrectly teaches that a mesophilic helicase could be used for the step of unwinding a duplex in amplification by applying the reaction conditions described in the Kuhn reference and in the Radding reference.

> The reaction conditions suitable for separating the strands of nucleic acid with helicases are described by Kuhn ....." (col. 12, lines 44-49)

These reaction conditions could not achieve nucleic acid amplification using helicases. Moreover, this use is not taught by the references.

The Radding reference (Annual Review of Genetics)

Rec A is a single strand binding protein. It is not capable of unwinding a duplex required in element (d) of claim 1. Instead, recA binds to a D-loop, which is a single strand region of a duplex and not the duplex itself. The recA protein:

> enables a single strand to find it's complement in duplex DNA more rapidly than it can find its free complement in solution under optimal conditions. (Radding p. 412)

> The prototype of the reaction catalyzed by RecA protein is the pairing of a linear single strand with duplex DNA to form a D-loop in which some part of the original single strand has become part of the helix, forming a heteroduplex region and displacing its homolog. (Radding p. 407)

> ...purified RecA protein, using the energy of ATP promotes homologous pairing of duplex molecules with single stranded or partially single stranded DNA. (Radding p. 407)

The claimed method requires the use of a helicase preparation for unwinding a duplex for purposes of amplification. The reaction conditions described in the Radding reference are not applicable to the claimed method.

Kuhn et al reference (CSH)

The reference describes RecBC and then teaches away from the use of this helicase for studies on unwinding of short duplex DNA fragments because the reference teaches away from a single strand binding protein.

The reference describes the use of three alternative mesophilic helicases for unwinding short duplex DNA fragments - DNA helicase I, UVrD helicase and T4 helicase for studies on DNA unwinding and ATP phosphorylation. The reference states (para. 1 line 16) that "The three enzymes discussed here are capable of dissociating a duplex completely into single strands independently of DNA binding proteins".

The reference describes the use of these three helicases with Exonuclease III to create a region of unpaired DNA to initiate unwinding (pg 64, left column, bottom of

page). Exonuclease III cannot be used in the claimed method of amplification because of its DNA degradation activity.

In contrast to the Kuhn reference, the applicants have found that these mesophilic helicases require single strand binding proteins for unwinding the duplex for purposes of amplification.

In summary, whereas the Examiner has rejected the claims of the above application as anticipated by Sninsky et al. under 35 U.S.C. §102(b) or obvious under 35 U.S.C. §103, the references neither teach nor enable the present claimed invention. It would not have been obvious to persons of ordinary skill in the art at the time of filing the application to practice the claimed invention after reading Sninsky et al. because Sninsky did not suggest or enable the use of a helicase preparation containing a single strand binding protein for unwinding the duplex for purposes of amplification.

Dean Patent: US 6,997,148 B2

The Examiner has pointed out numerous cites in this reference most of which provide background information for multiple displacement amplification, which generally relies on phage 29 DNA polymerase. The Examiner has cited col. 5, lines 55-67, col. 6 , lines 10-28, col. 7, lines 49-51, col. 8 lines 2-19, col. 26, lines 50-55, col .34, lines 38-51, col. 23, lines

50-57, col. 24, lines 16-49, 63-67, col. 25, lines 1-20, 53-60, col. 30 lines 35-48, 49-64, col. 36, lines 24-51, col. 37, lines 57-67 and col. 38, lines 1-19, 49-65. However, only column 24 recites the use of a helicase. However, the reference teaches the use of a helicase **or** a single strand binding protein in column 24, lines 17- 50 not both helicase and single stranded binding protein.

Dean et al. teaches the use of a mesophilic helicase **or** single strand binding protein for multiple displacement amplification

The reference teaches that a suitable temperature is 32°C stating: " For example, a helicase can be used instead of a SSB [single strand binding protein]. Such assays should be performed at a temperature suitable for optimal activity for the enzyme being used, for example 32°C" (column 24, line 44). This is compatible with the use of a mesophilic helicase, which is implied by the description. Nonetheless, there is no recognition of the requirement of a mesophilic helicase preparation containing a single strand binding protein not merely a helicase.

The reference states in column 24, lines 17-55 that a helicase can be used instead of SSB and describes the use of calf thymus helicase. However, calf thymus helicase is a mesophilic helicase and as such cannot be used in the absence of a SSB to unwind duplex for purposes of

amplification. Moreover, a single strand binding protein cannot on its own unwind a duplex nucleic acid.

Therefore, addition of this helicase to a reaction with a polymerase and primers as described in the Dean patent would not result in amplification.

In summary, while the Examiner has rejected the claims under 35 U.S.C. §102(e) as anticipated by the Dean reference, the Dean reference neither enables or teaches the claimed method of helicase dependent amplification.

CONCLUSION

For the reasons set forth above, Applicants respectfully submit that this case is in condition for immediate allowance. Early and favorable consideration leading to prompt issuance of this Application is earnestly solicited.

Applicants do not believe that any fees are due. However, please charge Deposit Account No. 14-0740 for any deficiencies.

Respectfully submitted,

NEW ENGLAND BIOLABS, INC.

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Harriet M. Strimpel D.Phil.
(Reg. No.: 37,008)
Attorney for Applicant
240 Country Road
Ipswich, Massachusetts 01938
(978) 380-7373

Customer No.: 28986